Effects of technological treatment on DNA degradation in selected food matrices of plant origin

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SUMMARY

Effects of thermal degradation on amplifiability and quantity of DNA in raw and processed food products were studied. Crucial step in DNA extraction from food samples is homogenization and heat treatment. Agarose gel electrophoresis and PCR methods were used to assess the extent of DNA degradation. Treatment by elevated temperature (200 °C) considerably reduced the size of extracted DNA. To assess the length of amplifiable DNA, relevant primers targeting genes specific for soya, beans, maize and pea were designed. Small DNA fragments with size ranging from 100 to 200 bp were amplified in all samples. Treatment at 80 °C had no effect on amplification of 1 371 bp bean, 1 325 bp maize, 913 bp soya and 874 bp pea DNA fragments. DNA fragments longer than 1 kbp were amplified only if heating at 100 °C and 200 °C lasted no longer than 60 min and 30 min, respectively. Higher temperature (220 °C) reduced the size of DNA extracted from bread so that fragments of soya lectin and epsps genes (both of 1 100 bp) could be no longer amplified. Similar results were obtained using maize genes where no amplification occurred for 1 000 bp amplicons of HMG and zein genes, respectively.

Keywords
DNA integrity; PCR; thermal treatment; soya; beans; maize; pea

Thermal treatment represents one of the most important technological processes in food production. Occurrence of pathogenic bacteria, allergenic species, deliberate food adulteration in such food or simply incorrect labelling (e.g. lack of declaration of allergens or genetically modified organisms (GMO) contents) may have adverse effects or mislead consumers, and is regulated [1-7]. Processing of food causes DNA degradation and may affect DNA-based food analysis [8-12, 13, 14]. Some aspects of quantification of DNA from GMO in composite and processed foods have been reviewed by ENGEL and co-workers [15].

Important source of proteins in food and feed are legumes. Beans and pea are an alternative source of protein to soya, and moreover are easily locally produced in Slovak Republic locally. Recently, maize and soya are the most frequently used genetically modified plants [16]. The detection of food components is mostly based on chemical characteristics, but typical proteins or DNA may be also used as analytical targets. The method of choice for DNA detection and quantification is polymerase chain reaction (PCR).

In our study, effects of the particle size and of the thermal treatment on DNA degradation in legumes and maize is presented, as visualised by agarose gel electrophoresis and confirmed by PCR.

MATERIAL AND METHODS

DNA degradation – thermal treatment

Seeds of pea (*Pisum sativum*, Gloriosa), beans (*Phaesolus vulgaris* L.), maize (*Zea mays* L., MON810) and soya (*Glycine max* L., Roundup Ready (RR), Merrill) were subjected to heat processing (boiling and baking) at 80 °C, 100 °C and 200 °C (Tab. 1) starting from laboratory temperature of water and prehetated oven to 200 °C, respectively. Samples were taken in 15 min inter-

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Tab. 1. Food matrices from soya, beans, pea and maize.

Product	Producer
Soy seeds	Marianna, Ivanka pri Dunaji, Slovakia
Soy cubes	AlfaBio Slovakia, Zvolen, Slovakia
Soy granule	Pragosója, Praha, Czech Republic
Soy flake	Marianna, Ivanka pri Dunaji, Slovakia
Soy flour	Sójaprodukt, Drietoma, Slovakia
Soy milk	Zajíc Plus, Zlín, Czech Republic
Tofu	AlfaBio, Banská Bystrica, Slovakia
Roast soy	M. M. Sója, Hronská Dúbrava, Slovakia
Pea Gloriosa	Lagris, Bratislava, Slovakia
Bean	Marianna, Ivanka pri Dunaji, Slovakia
Maize	Vince and Kroner, Bratislava, Slovakia
RR soy	Central Controlling and Testing Institute in Agriculture, Bratislava, Slovakia
Maize MON810	Agrokomplex, Kunovice, Czech Republic

Tab. 2. Effects of temperature, time and amplicon size on PCR amplification of DNA.

Treated samples/min	0	15	30	45	60	90	120	180	240	360
Soy (913 bp) boiled at 80 °C	+	+	+	+	+	+	+	+	+	+
Soy (913 bp) boiled at 100 °C	+	+	+	+	+	–	–	–	–	–
Pea (748 bp) baked at 100 °C	+	+	+	+	+	+	+	+	+	–
Pea (748 bp) baked at 200 °C	+	+	+	+	–	–	–	–	–	–

+ presence of amplicon, – absence of amplicon.

vals till 360 min at 80 °C and 100 °C, till 120 min at 200 °C (pea, maize) and till 75 min at 200 °C (beans) and analysed by electrophoresis in 1% agarose gel.

PCR analysis was performed after the samples were boiled in distilled water (pH 7.0) at 80 °C, 100 °C, and baked at 200 °C in the hot dry oven (FN 032-FN-55120, Nüve, Ankara, Turkey). The time intervals for sampling were every 15 min starting from 0 min (untreated) to 60 min followed by 30 min and 60 min intervals till 360 min (Tab. 2).

DNA degradation after baking at 220 °C (prehated oven) of the experimentally prepared small loaf of bread (wet weight approx. 60 g) was studied. The Canadian short advanced (CSP) recipe for bread was used [17]. The defined amount of RR soya flour was added to the dough. The mixture contained 35 g of wheat flour, 1.4 g saccharose, 0.2 g malt, 0.84 g table salt, 1 g of cooking margarine, 0.7 g instant yeast, 0.035 g of ammonium phosphate, 0.0053 g ascorbic acid and 21.7 ml of drinking water. PCR analysis was performed 30 min after the end of baking. Samples were taken from the top, bottom and middle of the bread loaf.

DNA extraction

Seed and food samples were homogenized in AY47R1 mixer (Moulinex, Barcelona, Spain). DNA was than extracted by the cetyl trimethylammonium bromide (CTAB) method [16] or GeneSpin kit (GeneScan, Teltow, Germany) [15]. DNA concentration was determined spectrophotometrically (SmartSpec Plus spectrophotometer, Bio-Rad, Hercules, California, USA), final volume of DNA solution was set to 100 μl. Integrity of DNA was determined electrophoretically in an agarose gel (1.5%) and documented by a digital camera PowerShot G5 (Canon, Tokyo, Japan).

Monitoring of DNA thermal degradation

PCR was performed in 25 μl volumes using GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA) or iCycler iQ (Bio-Rad, Sergate, Italy). The protocols for PCR are

Tab. 3. PCR programme for amplification of all used genes.

Step		Temperature	Time
Initial denaturation		95 °C	15 min
40 cycles	denaturation	95 °C	30 s
	annealing	64 °C	30 s
	extension	72 °C	40 s
Final extension		72 °C	3 min
Cooling		4 °C	∞

summarized in Tab. 3. Reaction mixture consisted of 1× concentrated PCR buffer (Qiagen, Hilden, Germany); 1.5 mmol.l^{-1} $MgCl_2$; 0.05% Tween 20; 200 µmol.l^{-1} dNTP (Invitrogen, Carlsbad, California, USA); 50 pmol.l^{-1} primers as presented (Tab. 4); 1 U HotStar Taq polymerase (Qiagen) and 2 µl of DNA solution. The sequences in GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA; with accession numbers in brackets) for pea lectin gene (X66368), bean lectin gene (AJ439563), bean phaseolin gene (J01263), soya lectin (AY342212), epsps gene (AJ783418), maize HMG (X72692) and maize zein gene (AF465640) were used for

Tab. 4. Used primers.

Primer	Sequence (5´→3´)	Size of PCR products	Reference
Pea-F3 Pea-R1	ttgtcataaatgcacccaacagttacaacg catactctgcgctattgaaaactcgaa	122 bp	this paper
Pea-F2 Pea-R1	atggcttctcttcaaacccaaatgatctcg catactctgcgctattgaaaactccgag	417 bp	this paper
Pea-F2 Pea-R2	atggcttctcttcaaacccaaatgatctcg gcatattctgctcctgtggtagctgag	748 bp	this paper
Pea-F3 Pea-R3	ttgtcataaatgcacccaacagttacaacg ccaaaatgttgagaggtgcacatgaacc	874 bp	this paper
Faz-F2 Faz-R2	cagtagacctgaagagcgttcttcc cggagagcttggaagcaaaagacc	116 bp	this paper
Faz-F1 Faz-R1	ctgatggagttcacgtcgatgcc ctcttccttgtgcttctcaccc	469 bp	this paper
Faz-F1 Faz-R2	cctcttccttgtgcttctcaccc cggagagcttggaagcaaaagacc	724 bp	this paper
Psn-F Psn-R	tcgtcttggtgaaacctgat ttttgctgttcctgttggtg	1371 bp	this paper
SoyGluSyn-F SoyGluSyn-R	gaggatcaccgagattgcaggag gaatggttgtctcagcaatcatggaag	913 bp	this paper
Soy L2-F Soy L2-R	gtgctactgaccagcaaggca ctgctagcgtgtggcaaattg	737 bp	this paper
SoyL1-3R	ctttcccgaggaggtcacaa	120 bp	this paper
SLe1-F SLe2-R	tgggacaaagaaaccggtag gtcaaactcaacagcgacga	201 bp	this paper
RRsoyaEv-F RRsoyaEv-R	ttcattcaaaataagatcatacatacaggtt ggcatttgtaggagccactt	84 bp	16
RRsoyaRef-F RRsoyaRef-R	ccagcttcgccgcttccttc gaaggcaagcccatctgcaagcc	78 bp	16
lvr1 F-A lvr1 R-B	ccgctgtatcacaagggctggtacc ggagcccgtgtagagcatgacgatc	224 bp	this paper
lvr1 F-I lvr1 R-B	tcctccactggctgcacctaccg ggagcccgtgtagagcatgacgatc	124 bp	this paper
lvr1 F-A lvr1 R-C	ccgctgtatcacaagggctggtacc cgtaggtgccgatcgcgtagtagtc	69 bp	this paper
lvr1 F-E lvr1 F-C	agtgggtcaagtcggacgccaacc cgtaggtgccgatcgcgtagtagtc	401 bp	this paper
lvr1 F-A lvr1 R-D	ccgctgtatcacaagggctggtacc aggatcggggcctctctgcttgaac	1339 bp	this paper
MON810-F MON810-R	tcgaaggacgaaggactctaacgt gccaccttccttttccactatctt	92 bp	16
HMG-F HMG-R	gctacatagggagccttgtcct ttggactagaaatctcgtgctga	79 bp	16
Lec-F Lec-R	gaggatcaccgagattgcaggag gaatggttgtctcagcaatcatggaag	1100 bp	this paper
Epsps-F Epsps-R	ccgcaaccgcccgcaaatcctct tcgccctcatcgcaatccacgcc	1100 bp	this paper

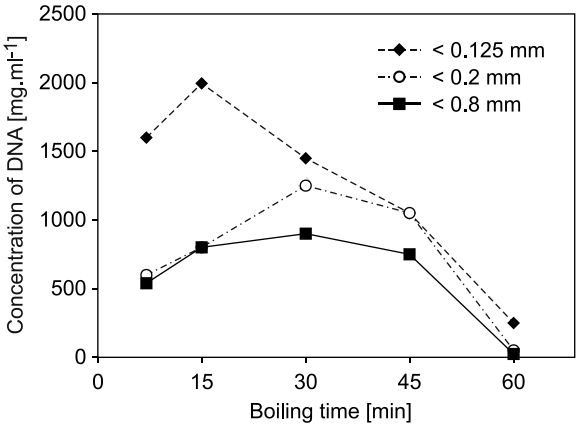

Fig. 1. The effect of flour particle size and thermal treatment on DNA extractability.

primers design, which was performed by the program Primer3 (Whitehead Institute Nine Cambridge Center, Cambridge, Massachusetts, USA). Amplified DNA fragments were analysed by electrophoresis in 1.5% agarose gel.

RESULTS AND DISCUSSION

Two different methods were used for genomic DNA extraction. The CTAB method and GeneSpin kit showed different effectiveness for treated food matrices. Higher yields of extracted DNA were obtained with the GeneSpin kit. The CTAB method was more appropriate for the extraction of DNA from processed soya (e.g. granules), while the GeneSpin kit was more effective for the extraction of DNA from raw materials (data not shown).

The effect of flour particle size and thermal treatment on the amount of extracted DNA is presented in Fig. 1. The extractability of DNA increased by time of boiling reaching a maximum of 2000 μg.ml^{-1} after 15 min for 0.125 mm particle size. For larger particles (0.2–0.8 mm), the optimal extraction time was 30 min with DNA yields of 800–1200 μg.ml^{-1}. We speculate that better extractability was due to partial degradation of high molecular weight DNA at the beginning of boiling followed by further degradation after 60 min of boiling, when the amount of extracted DNA decreased to less than 200 μg.ml^{-1}.

The degree of DNA degradation by heat processing was monitored by agarose gel electrophoresis and PCR as presented in the examples at Fig. 2–4. Maize, bean, soya and pea were boiled in distilled water at 80 °C, 100 °C and 200 °C and oven-baked at 100 °C and 200 °C. Higher temperatures (100 °C and 200 °C) considerably reduced the size of the extracted DNA in a time-dependent manner. The temperature-dependent DNA degradation was monitored using PCR. The degree of DNA degradation was determined by PCR amplification applying primers in a way that amplicons of different sizes were obtained. In this way, large DNA fragments invisible on agarose gels were amplified, too.

The average size of prokaryotic structural genes ranges about 1000 bp so the primers for DNA fragment amplification were designed in this and/or smaller sizes. The PCR systems were designed to amplify five maize, four of each bean, pea and soya amplicons.

The level of DNA degradation in soya samples boiled and baked at a temperature of 100 °C or lower was not fully completed after 240 min, while baking at 200 °C significantly decreased the time necessary for DNA degradation (Fig. 3, 4). The amplification of the 1379-bp DNA fragment

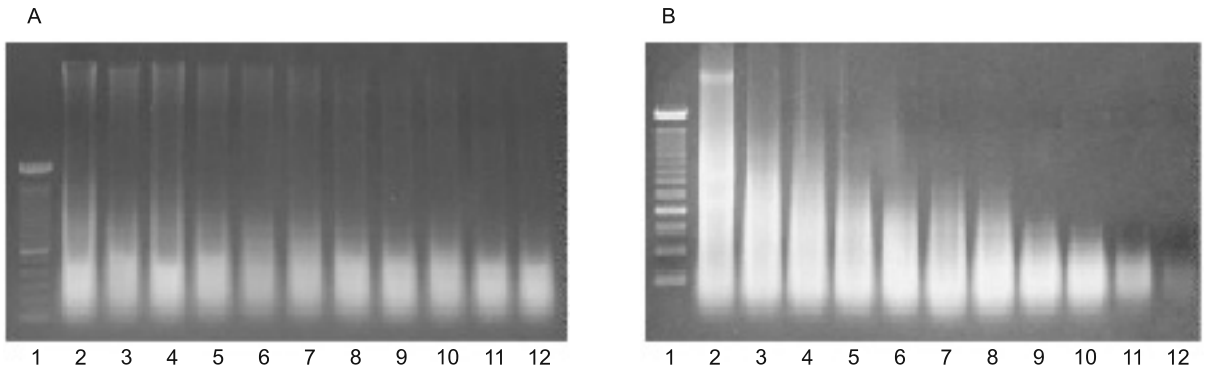

Fig. 2. The level of DNA degradation in heat-processed pea samples.
A - at 80 °C, 1 - standard *n* × 250 bp, B - at 100 °C, 1 - standard *n* × 100 bp, 2–12 - (0, 7, 15, 30, 45, 60, 90, 120, 150, 225, 360 min).

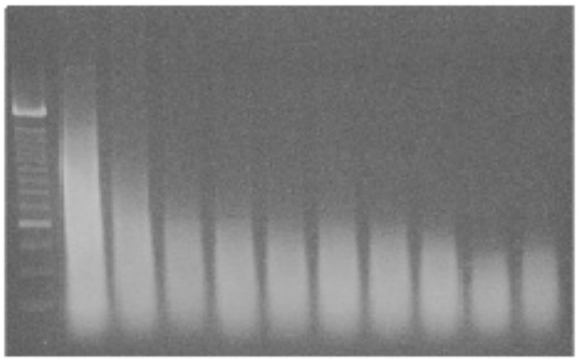

Fig. 3. Time-dependend DNA degradation in mild roasted soya (100 °C).
1 - standard n × 250 bp, 2 - 0 min, 3 - 7 min, 4 - 15 min, 5 - 30 min, 6 - 45 min, 7 – 60 min, 8 - 90 min, 9 - 120 min, 10 - 150 min, 11 - 225 min.

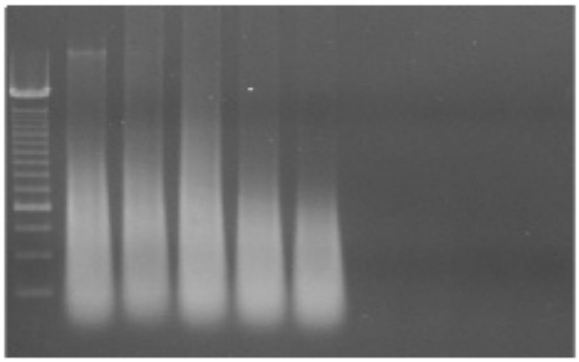

Fig. 4. Time-dependend DNA degradation in roasted soya (200 °C).
1 - standard n × 250 bp, 2 - 0 min, 3 - 7 min, 4 - 15 min, 5 - 30 min, 6 - 45 min, 7 – 60 min, 8 - 75 min, 9 - 90 min, 10 - 120 min.

(phaseolin gene) failed after 45 min of incubation, and amplification of the 1339-bp DNA fragment (invertase gene) failed after 15 min when heated at 100 °C and 200 °C. The 913 bp amplicons were detectable during 6 h of heating at 80 °C (Tab. 2), while only during 45 min of heating at 100 °C (Tab. 2). Similar results were obtained for thermally treated pea samples (Tab. 2). BAUER and co-workers [14] have shown that a combination of treatments has a more pronounced effect. In their experiment, a 1225 bp long DNA fragment from maize Bt-176 was no longer detectable after a 30-min incubation at pH 4.0 and 65 °C.

Degradation of DNA by heat processing in experimentally prepared small loaves of bread was monitored using added soya and maize and baked at 100 °C or 220 °C. The samples were taken from different parts of breads: from the top crust, the bottom crust and from the middle of the bread. Integrity of DNA was evaluated by agarose gel electrophoresis and PCR analysis (Fig. 5). Baking temperature (220 °C) considerably reduced the size of the extracted amplifiable DNA as shown on amplicons for lectin and for epsps gene (both of 1100 bp; Fig. 5) in the top crust of the soya-containing bread. Comparable results were obtained with maize flour where no amplification occurred for 1000 bp amplicons of HMG and zein genes, respectively. The explanation could be that the heat transfer is better on the surface of the loaf, while the core contains more moisture and is generally less heated [8, 12, 16].

Effects of DNA degradation on DNA amplification of commercially available foods showed analogous results as those for experimental samples. Processed soya food matrices (soya cubes, soya granules, soya milk, tofu, soya flakes and roasted soya) as listed in Tab. 1 were tested. In some matrices (soya cubes, granules, flakes and tofu), the 913 bp DNA fragment was amplified, while in soya milk and roasted soya products, small DNA fragments (120 bp and 201 bp) were detected only. This corresponds to earlier data [10, 12, 13, 18] and clearly indicates that for all processed food matrices, only DNA fragments of 120–200 bp are generally best candidates for PCR analysis.

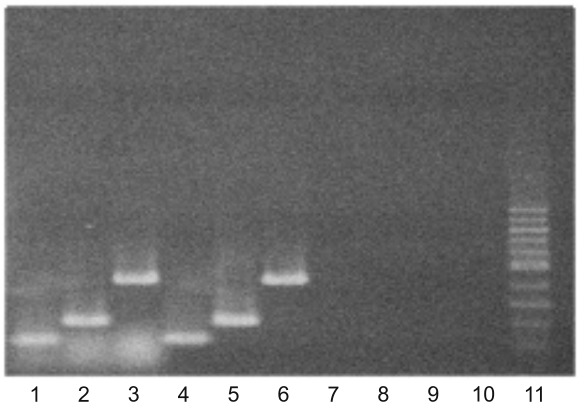

Fig. 5. DNA degradation of lectine gene of RR soya baked at 220 °C.
1 - DNA fragment 120 bp, 2 - DNA fragment 201 bp, 3 - DNA fragment 410 bp, 4–6 - positive control for DNA fragments 120 bp, 201 bp, 410 bp, 7 - DNA fragment 1100 bp, 8–10 - negative control, 11 - standard n × 100 bp.

CONCLUSIONS

The effect of heat processing parameters on the degradation of plant DNA in food was monitored by PCR. Matrix particle size and time-dependent thermal treatment have a significant effect on the amount of DNA extracted from food matrix. The size of amplicons obtained has shown similar temperature and time dependency for all studied matrices. It was shown that the integrity of heated DNA decreased in correspondence to the duration of the exposure. Maximum size of PCR products, that were amplifiable, reflected the level of DNA degradation due to processing. The particle size of flour, degree and duration of thermal treatment, may negatively influence the detection or quantification of food components after technological processing of foods. Generally, the amplicon size should be around 200 bp for analysis of processed foods by PCR.

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